# Comparison of bloodstream fatty acid composition from African-American women at gestation, delivery, and postpartum<sup>®</sup>

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Abstract Our aim was to examine the docosahexaenoic acid (DHA; 22:6n-3) status of pregnant African-American women reporting to the antenatal clinic at Wayne State University in a longitudinal study design. Fatty acid compositions of plasma and erythrocyte total lipid extracts were determined and food frequency surveys were administered at 24 weeks of gestation, delivery, and 3 months postpartum for participants (n = 157). DHA (mean  $\pm$  SD) in the estimated total circulating plasma was similar at gestation  $(384 \pm 162 \text{ mg})$  and delivery  $(372 \pm 155 \text{ mg})$  but was significantly lower at 3 months postpartum (178  $\pm$  81 mg). The relative weight percentage of DHA and docosapentaenoic acid n-6 (DPAn-6; 22:5n-6) decreased postpartum, whereas their respective metabolic precursors, eicosapentaenoic acid (EPA; 20:5n-3) and arachidonic acid (AA; 20:4n-6), increased. Similar results were found in erythrocytes. Dietary intake of DHA throughout the study was estimated at  $68 \pm 75$  mg/day. The relative amounts of circulating DHA and DPAn-6 were increased during pregnancy compared with 3 months postpartum, possibly via increased synthesis from EPA and AA. The low dietary intake and blood levels of DHA in this population compared with others may not support optimal fetal DHA accretion and subsequent neural development.—Stark, K. D., S. Beblo, M. Murthy, M. Buda-Abela, J. Janisse, H. Rockett, J. E. Whitty, S. S. Martier, R. J. Sokol, J. H. Hannigan, and N. Salem, Jr. Comparison of bloodstream fatty acid composition from African-American women at gestation, delivery, and postpartum. J. Lipid Res. 2005. 46: 516-525.

**Supplementary key words** n-3 fatty acids • omega-3 • docosa-hexaenoic acid • arachidonic acid • pregnancy • dietary intake • maternal nutrition

The importance of docosahexaenoic acid (DHA; 22:6n-3)

Manuscript received 8 October 2004 and in revised form 8 December 2004. Published, JLR Papers in Press, December 16, 2004. DOI 10.1194/jlr.M400394-JLR200 for optimal infant neural and retinal development and its mechanisms of action have been reviewed (1–3). Low DHA intake in infants is associated with decreased visual acuity (4–7), lower cognitive scores (8, 9), and poorer performance on multipart problem-solving tasks (10). Also, low consumption of fish is an identified risk factor for preterm delivery and low infant birth weight (11), whereas maternal DHA supplementation increases gestational length (12). A low dietary intake of n-3 PUFAs, and of DHA in particular, is associated with a higher risk for postpartum maternal depression (13, 14).

The importance of fatty acids in infant development has led to several studies examining fatty acid status in women during pregnancy (15–20), comparisons of fatty acid status in pregnant versus nonpregnant women (21–24), and studies of postpartum fatty acid status (22, 25–28). Plasma lipid concentrations increase most rapidly in the early stages of pregnancy and plateau (29). For this reason, fatty acid status during pregnancy is best reported as both a relative percentage and an absolute concentration for proper interpretation. Because there is also a significant and constant expansion of blood and particularly plasma volume throughout pregnancy (30–32), reporting absolute amounts of fatty acids in the total circulating pool is necessary for a more complete understanding of maternal fatty acid status.

A number of studies have reported the fatty acid composition of the phospholipid fraction during pregnancy. The relative percentage and the absolute concentration of DHA

Abbreviations: AA, arachidonic acid; DHA, docosahexaenoic acid; DPAn-3, docosapentaenoic acid n-3; DPAn-6, docosapentaenoic acid n-6; EPA, eicosapentaenoic acid; HUFA, highly unsaturated fatty acid.

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The online version of this article (available at http://www.jlr.org) contains an additional three tables.

TABLE 1. Characteristics of African-American women at entry into the study

Variable	Value
Age (years)	$24.5 \pm 5.2$
Height (m)	$1.65 \pm 0.07$
Prepregnancy body weight (kg)	$75.1 \pm 22.5$
Prepregnancy body mass index (kg/m <sup>2</sup> )	$27.6 \pm 7.9$
Body weight at first prenatal visit (kg)	$82.2 \pm 22.7$
Body mass index at first prenatal visit (kg/m <sup>2</sup> )	$30.1 \pm 7.8$
Education (highest grade)	$11.8 \pm 1.3$
Blood glucose at first prenatal visit (mmol/l)	$4.55 \pm 0.45$
Socioeconomic status (Hollingshead class)	$3.9 \pm 1.1$
Total number of pregnancies	$3.5 \pm 2.1$
Total number of deliveries	$1.5 \pm 1.5$
Smoking (cigarettes/day)	$4.5 \pm 7.2$

Values are means  $\pm$  SD (n = 157). No significant differences were detected between samples at each time point by one-way ANOVA.

in plasma phospholipid increase during early pregnancy (15, 24). In later pregnancy, although the relative percentage of DHA decreases, the concentration of DHA remains stable, or possibly increases (15, 20). There is a rapid postpartum decrease in both the relative percentage and absolute concentrations of phospholipid DHA (25, 27, 28). Although phospholipid fatty acids represent structural lipids

and are biomarkers for dietary fatty acid intake (33), this fraction may not be the best indicator of fatty acid availability for placental transfer. Transfer across the placenta is limited to nonesterified fatty acids, and the localization of lipoprotein lipase and triacylglycerol hydrolase activities (34) to the placenta has led to suggestions that triacylglycerols are the major source of maternal fatty acids (35, 36), although mobilization from other circulating lipid classes is also possible (21). The selectivity of nonesterified fatty acid uptake is likely controlled by cytoplasmic fatty acid binding protein, fatty acid translocase, fatty acid transporter protein, and plasma membrane fatty acid binding protein (37).

In the present study, fatty acid compositional analyses of plasma and erythrocyte total lipid extracts and dietary intake analysis of food frequency surveys from inner-city, pregnant African-American women were completed at 24 weeks of gestation, at delivery, and at 3 months postpartum to determine DHA status. As far as we are aware, this is also the first study to examine the total amounts of individual plasma fatty acids with consideration of pregnancy-induced plasma volume expansion. This is also the largest study to date to examine circulating fatty acids during late pregnancy and postpartum and to determine and control for dietary intakes of individual fatty acids during

TABLE 2. Selected dietary intakes of pregnant African-American women at 24 weeks gestation, infant delivery, and 3 months postpartum

Variable	24 Weeks Gestation	Delivery	3 Months Postpartum
Protein (g)	$78.1 \pm 16.6^{a}$	$72.3 \pm 12.2^{b}$	$59.4 \pm 15.3^{c}$
% of energy	$13.1 \pm 3.0$	$13.1 \pm 2.4$	$12.9 \pm 3.1$
Carbohydrates (g)	$279 \pm 44^{a}$	$258 \pm 41^{b}$	$202 \pm 35^{c}$
% of energy	$47.1 \pm 7.9^a$	$46.1 \pm 7.6^{a}$	$43.6 \pm 8.2^{b}$
Fat (g)	$98 \pm 14^{a}$	$93 \pm 15^{b}$	$79 \pm 12^{c}$
% of energy	$38.2 \pm 5.8^{a}$	$39.3 \pm 6.2^{a,b}$	$40.8 \pm 7.2^{b}$
Saturated fat (g)	$38.4 \pm 7.7^{a}$	$36.7 \pm 6.7^{a}$	$30.2 \pm 6.7^{b}$
% of energy	$14.9 \pm 2.9$	$15.3 \pm 2.7$	$15.3 \pm 2.9$
14:0 (g)	$3.3 \pm 1.4^{a}$	$3.2 \pm 1.2^{a}$	$2.4 \pm 1.3^{b}$
16:0 (g)	$21.1 \pm 3.8^a$	$20.0 \pm 3.3^{b}$	$17.1 \pm 3.1^{c}$
18:0 (g)	$9.7 \pm 1.8^{a}$	$9.3 \pm 1.7^{a}$	$7.8 \pm 1.7^{b}$
Monounsaturated fat (g)	$40.2 \pm 6.4^{a}$	$38.6 \pm 6.8^{a}$	$33.0 \pm 5.6^{b}$
% of energy	$15.8 \pm 2.5^{a}$	$16.4 \pm 2.8^{a,b}$	$17.2 \pm 3.7^{b}$
16:1n-7 (g)	$1.8 \pm 0.5^{a}$	$1.7 \pm 0.4^{a}$	$1.5 \pm 0.5^{b}$
18:1n-9 (g)	$37.7 \pm 6.1^{a}$	$36.2 \pm 6.6^{a}$	$31.0 \pm 5.3^{b}$
20:1n-9 (mg)	$163 \pm 85^{a}$	$137 \pm 65^{b}$	$146 \pm 76^{a,b}$
Polyunsaturated fat (g)	$19.0 \pm 4.8^{a}$	$17.7 \pm 4.4^{b}$	$16.0 \pm 4.2^{c}$
% of energy	$7.5 \pm 1.9^{a}$	$7.6 \pm 2.0^{a}$	$8.4 \pm 2.2^{b}$
n-6 PUFA (g)	$16.9 \pm 4.5^{a}$	$15.7 \pm 4.1^{b}$	$14.3 \pm 4.0^{c}$
18:2n-6 (g)	$16.8 \pm 4.4^{a}$	$15.6 \pm 4.1^{b}$	$14.2 \pm 4.0^{c}$
20:4n-6 (mg)	$115 \pm 63$	$102 \pm 67$	$106 \pm 84$
22:5n-6 (mg)	$13.0 \pm 13.2$	$9.5 \pm 9.3$	$11.3 \pm 12.2$
n-6 HUFA (mg)	$128 \pm 71$	$112 \pm 72$	$117 \pm 91$
n-3 PUFA (g)	$1.80 \pm 0.42^{a}$	$1.63 \pm 0.34^{b}$	$1.46 \pm 0.38^{c}$
18:3n-3 (g)	$1.68 \pm 0.41^{a}$	$1.55 \pm 0.32^{b}$	$1.36 \pm 0.35^{c}$
20:5n-3 (mg)	$38 \pm 61$	$26 \pm 42$	$28 \pm 42$
22:6n-3 (mg)	$81 \pm 94$	$59 \pm 59$	$65 \pm 67$
n-3 HUFA (mg)	$119 \pm 152$	$85 \pm 100$	$93 \pm 107$
Dietary cholesterol (mg)	$298 \pm 99^{a}$	$280 \pm 88^{a}$	$236 \pm 96^{b}$
Vitamin A (IU)	$7,713 \pm 5,770^a$	$6,282 \pm 3,973^b$	$4,920 \pm 3,047^{c}$
Vitamin C (mg)	$149 \pm 106^{a}$	$131 \pm 82^{a}$	$90 \pm 64^{b}$
Vitamin E (IU)	$10.9 \pm 2.7^{a}$	$9.7 \pm 2.3^{b}$	$9.4 \pm 2.3^{b}$
Total energy (MJ)	$9.5 \pm 4.8^{a}$	$9.1 \pm 4.1^{a}$	$7.6 \pm 3.8^{b}$

Values are means  $\pm$  SD (n = 157). Dietary values are adjusted for energy by the nutrient residual model (43). HUFA, highly unsaturated fatty acid ( $\geq$ 20 carbons and  $\geq$ 3 double bonds).

a,b,c Means with different superscripts are significantly different across the three time points by Bonferroni post hoc tests after significance was detected by the linear mixed models procedure (P < 0.01).

TABLE 3. Plasma fatty acid composition at 24 weeks gestation, infant delivery, and 3 months postpartum expressed as a percentage

Fatty Acids	24 Weeks Gestation	Delivery	3 Months Postpartum		
	% by weight of total fatty acids				
$SFA^a$	$31.07 \pm 2.40^{b}$	$31.88 \pm 2.30^{c}$	$30.92 \pm 2.03^b$		
$14:0^{a}$	$0.89 \pm 0.34^{b}$	$0.68 \pm 0.22^{c}$	$0.75 \pm 0.27^d$		
$16:0^{a}$	$22.69 \pm 1.96^{b}$	$23.87 \pm 2.11^{c}$	$20.35 \pm 1.57^d$		
$18:0^{a}$	$6.25 \pm 0.80^{b}$	$6.09 \pm 0.82^{b}$	$8.27 \pm 0.97^{c}$		
20:0	$0.23 \pm 0.08^{b}$	$0.20 \pm 0.04^{c}$	$0.22 \pm 0.04^{b,c}$		
22:0	$0.45 \pm 0.11^{b}$	$0.56 \pm 0.13^{c}$	$0.67 \pm 0.16^d$		
24:0	$0.36 \pm 0.12^{b}$	$0.42 \pm 0.13^{c}$	$0.52 \pm 0.12^d$		
$MUFA^a$	$24.05 \pm 2.41^{b}$	$25.58 \pm 2.52^{c}$	$21.40 \pm 2.45^d$		
$16:1n-7^a$	$2.09 \pm 0.74^{b}$	$1.96 \pm 0.62^{b}$	$1.68 \pm 0.49^{c}$		
18:1n-7	$2.26 \pm 0.31$	$2.23 \pm 0.30$	$2.27 \pm 0.37$		
$18:1n-9^a$	$17.67 \pm 1.92^{b}$	$19.47 \pm 2.25^{c}$	$15.44 \pm 2.03^d$		
$20:1n-9^{a}$	$0.16 \pm 0.06^{b}$	$0.22 \pm 0.06^{c}$	$0.17 \pm 0.05^{b}$		
22:1n-9	$0.05 \pm 0.03^{b}$	$0.04 \pm 0.01^{b}$	$0.08 \pm 0.03^{c}$		
24:1n-9	$1.70 \pm 0.77$	$1.62 \pm 0.51$	$1.64 \pm 0.69$		
Total PUFA <sup>a</sup>	$39.51 \pm 4.01^{b}$	$37.62 \pm 3.43^{c}$	$42.25 \pm 3.34^d$		
n-6 PUFA <sup>a</sup>	$36.86 \pm 3.88^{b}$	$35.12 \pm 3.27^{c}$	$39.58 \pm 3.25^d$		
18:2n-6a	$26.54 \pm 3.74^{b}$	$25.12 \pm 3.26^{c}$	$27.57 \pm 3.24^d$		
18:3n-6	$0.19 \pm 0.07^{b}$	$0.17 \pm 0.06^{c}$	$0.40 \pm 0.15^d$		
20:2n-6	$0.27 \pm 0.05^{b}$	$0.26 \pm 0.05^{b}$	$0.21 \pm 0.05^{c}$		
20:3n-6	$1.45 \pm 0.27^{b}$	$1.46 \pm 0.31^{b}$	$1.66 \pm 0.37^{c}$		
20:4n-6a	$7.55 \pm 1.49^{b}$	$7.17 \pm 1.57^{b}$	$9.01 \pm 1.35^{c}$		
22:2n-6	$0.02 \pm 0.01^{b}$	$0.02 \pm 0.01^{b}$	$0.04 \pm 0.02^{c}$		
22:4n-6	$0.33 \pm 0.08^{b}$	$0.33 \pm 0.07^{b}$	$0.37 \pm 0.09^{c}$		
$22:5n-6^{a}$	$0.52 \pm 0.16^{b}$	$0.60 \pm 0.16^{c}$	$0.35 \pm 0.09^d$		
n-6 HUFA <sup>a</sup>	$9.86 \pm 1.63^{b}$	$9.56 \pm 1.82^{b}$	$11.40 \pm 1.50^{c}$		
n-3 PUFA <sup>e</sup>	$2.65 \pm 0.52^{b}$	$2.49 \pm 0.47^{c}$	$2.67 \pm 0.54^{b}$		
18:3n-3a	$0.44 \pm 0.15$	$0.41 \pm 0.11$	$0.42 \pm 0.15$		
20:3n-3	$0.019 \pm 0.008^{b}$	$0.016 \pm 0.007^{c}$	$0.047 \pm 0.035^d$		
20:5n-3 <sup>a</sup>	$0.17 \pm 0.07^{b}$	$0.15 \pm 0.05^{c}$	$0.41 \pm 0.19^d$		
22:5n-3	$0.25 \pm 0.07^{b}$	$0.25 \pm 0.06^{b}$	$0.44 \pm 0.10^{c}$		
22:6n-3 <sup>a</sup>	$1.77 \pm 0.44^{b}$	$1.68 \pm 0.40^{b}$	$1.37 \pm 0.37^{c}$		
n-3 HUFA <sup>a</sup>	$2.21 \pm 0.50^{b,c}$	$2.08 \pm 0.46^{b}$	$2.25 \pm 0.52^{c}$		
T . I I I I I I I I A A	$12.07 \pm 1.98^{b}$	$11.65 \pm 2.15^{b}$	$13.68 \pm 1.80^{\circ}$		

Values are means  $\pm$  SD (n = 157) for plasma total lipid extracts. SFA, saturated fatty acid.

pregnancy. In particular, the dietary intakes of DHA and arachidonic acid (AA; 20:4n-6), the predominant highly unsaturated fatty acids (HUFAs; ≥20 carbons and ≥3 double bonds) in the fetal brain, were determined. In addition, because of the large sample size and the analysis of both plasma and erythrocyte fatty acid compositions in the present study, proposed biomarkers of n-3 fatty acid status, including the relative percentage of eicosapentaenoic acid (EPA; 20:5n-3) plus DHA (38), and the percentage of n-3 HUFAs in total HUFAs (39) were examined.

## SUBJECTS AND METHODS

# Subjects and study design

All procedures and protocols received prior approval by the Wayne State University Human Investigations Committee, and informed consent was obtained during the initial clinic visit. Preg-

TABLE 4. Erythrocyte fatty acid composition at 24 weeks gestation, infant delivery, and 3 months postpartum expressed as a percentage

Fatty Acids	24 Weeks Gestation	Delivery	3 Months Postpartum
	% by :	weight of total fatty of	ıcids
$SFA^a$	$39.07 \pm 2.03^{b}$	$41.46 \pm 3.23^{c}$	$37.07 \pm 1.24$
$14:0^{a}$	$0.76 \pm 0.23$	$0.77 \pm 0.43$	$0.81 \pm 0.26$
$16:0^{a}$	$22.78 \pm 1.25^{b}$	$23.44 \pm 2.02^{c}$	$19.35 \pm 1.13$
$18:0^{a}$	$9.68 \pm 0.82^{b}$	$10.68 \pm 1.43^{c}$	$11.20 \pm 1.16$
20:0	$0.33 \pm 0.05^{b}$	$0.38 \pm 0.13^{c}$	$0.31 \pm 0.04$
22:0	$1.58 \pm 0.25^{b,c}$	$1.65 \pm 0.46^{b}$	$1.53 \pm 0.21$
24:0	$3.90 \pm 0.53^{b}$	$4.25 \pm 1.20^{c}$	$3.85 \pm 0.53$
$MUFA^a$	$19.72 \pm 1.26^{b}$	$20.02 \pm 1.98^{b}$	$21.00 \pm 1.42$
$16:1n-7^{a}$	$0.43 \pm 0.09^{b}$	$0.72 \pm 0.82^{c}$	$0.48 \pm 0.15$
18:1n-7	$1.81 \pm 0.19^{b}$	$1.80 \pm 0.27^{b,c}$	$1.73 \pm 0.24$
18:1n-9a	$10.57 \pm 0.75^{b}$	$11.31 \pm 1.10^{c}$	$10.15 \pm 0.78$
20:1n-9a	$0.25 \pm 0.07^{b}$	$0.30 \pm 0.15^{c}$	$0.22 \pm 0.05$
22:1n-9	$0.09 \pm 0.02^{b}$	$0.09 \pm 0.08^{b,c}$	$0.07 \pm 0.02$
24:1n-9	$6.31 \pm 1.01^{b}$	$5.50 \pm 1.60^{c}$	$7.89 \pm 1.43$
Total PUFA <sup>a</sup>	$34.54 \pm 1.31^{b}$	$35.10 \pm 2.61^{c}$	$36.12 \pm 1.28$
n-6 PUFA <sup>e</sup>	$28.85 \pm 1.17^{b}$	$29.32 \pm 2.15^{b}$	$30.71 \pm 1.23$
18:2n-6a	$9.22 \pm 0.89^{b}$	$9.56 \pm 1.47^{c}$	$9.75 \pm 0.99$
18:3n-6	$0.05 \pm 0.01$	$0.08 \pm 0.14$	$0.04 \pm 0.02$
20:2n-6	$0.31 \pm 0.04^{b}$	$0.26 \pm 0.10^{c}$	$0.27 \pm 0.04$
20:3n-6	$1.30 \pm 0.21^{b}$	$1.40 \pm 0.24^{c}$	$1.37 \pm 0.22$
20:4n-6a	$12.60 \pm 0.90^{b}$	$12.57 \pm 1.43^{b}$	$14.06 \pm 0.95$
22:2n-6	$0.07 \pm 0.02^{b}$	$0.13 \pm 0.11^{c}$	$0.08 \pm 0.02$
22:4n-6	$4.24 \pm 0.47$	$4.25 \pm 0.60$	$4.22 \pm 0.44$
22:5n-6a	$1.06 \pm 0.21^{b}$	$1.21 \pm 0.27^{c}$	$0.92 \pm 0.19$
n-6 HUFAa	$19.29 \pm 1.06^{b}$	$19.42 \pm 1.88^{b}$	$20.65 \pm 1.19$
n-3 PUFA <sup>a</sup>	$5.68 \pm 0.66^{b}$	$5.78 \pm 0.88^{b}$	$5.41 \pm 0.71$
18:3n-3a	$0.11 \pm 0.03^{b}$	$0.13 \pm 0.08^{c}$	$0.11 \pm 0.03$
20:3n-3	$0.04 \pm 0.01^{b}$	$0.10 \pm 0.13^{c}$	$0.04 \pm 0.02$
20:5n-3a	$0.19 \pm 0.05^{b}$	$0.21 \pm 0.18^{b}$	$0.35 \pm 0.12$
22:5n-3	$1.42 \pm 0.17^{b}$	$1.41 \pm 0.24^{b}$	$1.69 \pm 0.20$
22:6n-3a	$3.97 \pm 0.57^{b}$	$4.04 \pm 0.75^{b}$	$3.23 \pm 0.59$
n-3 HUFA <sup>a</sup>	$5.58 \pm 0.65^{b}$	$5.67 \pm 0.86^{b}$	$5.30 \pm 0.71$
Total HUFA <sup>a</sup>	$24.86 \pm 1.32^{b}$	$25.09 \pm 2.40^a$	$25.98 \pm 1.34$

Values are means  $\pm$  SD (n = 157) for the erythrocyte total lipid extracts.

 $^{b,c,d}$  Means with different superscripts are significantly different across the three time points by Bonferroni post hoc tests after significance was detected by the linear mixed models procedure (P < 0.01).

<sup>e</sup>Inclusion of dietary n-6 PUFA intake as a covariate resulted in an additional difference between the estimated marginal means of 24 weeks gestation and infant delivery by Bonferroni post hoc tests.

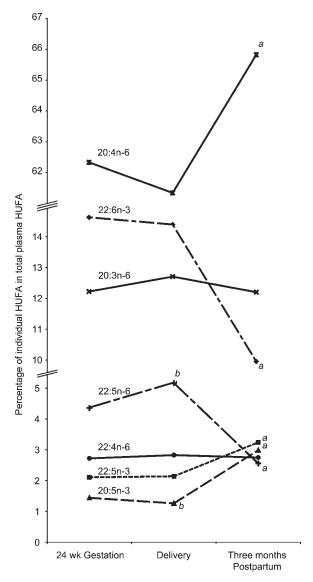
nant African-American women presenting at the Antenatal Clinic of Wayne State University (Detroit, MI) were recruited into the study. Women with known fatty acid metabolic disorders and with high-risk pregnancies, including hypertensives, diabetics, and those developing gestational diabetes, were excluded from the study. As alcohol has known effects on fatty acid metabolism and composition (40, 41), women consuming alcohol during pregnancy were not included in this analysis focused on pregnancy and postpartum effects. Maternal fasting blood samples (15 ml) were collected by venipuncture at 24 weeks of gestation, at delivery, and at 3 months postpartum. Specimens were collected into heparinized tubes, kept cold (4°C) until centrifuged (5 min at 2,000 g) to separate plasma and erythrocytes, and frozen at -75°C until analysis. Nutritional status at each time point was also assessed using a food frequency survey validated for low-income pregnant women (42) and modified to quantify selected dietary fats. Individual nutrient intakes were adjusted for total energy intake using the nutrient residual model (43). A modified Hollingshead index was used to measure socioeconomic status (44).

<sup>&</sup>lt;sup>a</sup> Fatty acids were also analyzed with the corresponding adjusted dietary intakes included as covariates in the model with no changes in the statistical results.

 $<sup>^{</sup>b,c,d}$  Means with different superscripts are significantly different across the three time points by Bonferroni post hoc tests after significance was detected by the linear mixed models procedure (P < 0.01).

<sup>&</sup>lt;sup>e</sup>Inclusion of dietary n-3 PUFA intake as a covariate resulted in no difference between the estimated marginal means of 24 weeks gestation and infant delivery by Bonferroni post hoc tests.

<sup>&</sup>lt;sup>a</sup>Fatty acids were also analyzed with the corresponding adjusted dietary intakes included as covariates in the model with no changes in the statistical results.



**Fig. 1.** Percentage of individual highly unsaturated fatty acids (HUFAs;  $\geq$ 20 carbons and  $\geq$ 3 double bonds) in total HUFAs in plasma. Each point represents the mean for plasma samples (n = 157) at each time point. Means were compared by Bonferroni post hoc tests after significance was detected by the linear mixed models procedure (P < 0.01). <sup>a</sup> Significantly different from 24 weeks (wk) gestation and delivery. <sup>b</sup> Significantly different from 24 weeks gestation and 3 months postpartum.

#### Laboratory analyses

Total lipids were extracted from plasma samples according to the method of Folch, Lees, and Stanley (45) and from erythrocytes according to the method of Reed et al. (46) with an internal standard (23:0 or 22:3n-3; NuCheck Prep, Elysian, MN). Lipid extracts were then methylated with boron trifluoride in methanol (14% w/v; Alltech Associates, Deerfield, IL) (47), and fatty acid methyl esters were collected and analyzed by capillary gas chromatography according to Salem, Reyzer, and Karanian (48) on an Agilent 6890N gas chromatograph (Agilent, Palo Alto, CA) with a 0.25 mm  $\times$  30 m DB-FFAP column (J & W Scientific, La Palma, CA). Fatty acid analyses were successfully completed on >1,200 blood samples. Participants with complete fatty acid compositional data at 24 weeks gestation, delivery, and 3 months postpar-

tum for both plasma and erythrocytes were included in the present study (n = 157). Plasma fatty acid analyses are presented as relative weight percentages, absolute concentrations ( $\mu$ g/ml), and estimated amounts in the entire plasma pool (mg). Erythrocytes are presented as relative weight percentages only, because accurate counts of erythrocytes were not completed at the time of sample collection. Individual basal plasma pool volumes were estimated by first determining prepregnancy plasma volume (45 ml plasma/kg), and then at each time point, relative to this basal level, current plasma volumes were estimated by adjusting for a 35% increase at 24 weeks gestation, a 50% increase at delivery, and a 2% increase at 3 months postpartum (30, 32).

#### Statistical analyses

All statistical analyses were completed with SPSS for Windows statistical software (release 11.5.1; SPPS, Inc., Chicago, IL). The linear mixed models procedure was used for repeated-measures analyses. Individual dietary intake of specific fatty acids was controlled for in these analyses by including the adjusted intake value of a specific fatty acid (when available) as a covariate (i.e., adjusted DHA intake was included as a covariate when DHA in either plasma or erythrocytes was examined as the dependent variable). This approach allowed for increased confidence that the observed differences at 24 weeks gestation, delivery, and 3 months postpartum are not the result of changes in dietary intakes during pregnancy. However, it does not control for the influence of the dietary intake of one fatty acid on the blood status of another. To reduce the risk of α slippage with multiple testing, significance was set at P < 0.01 and Bonferroni post hoc tests were used to control for multiple comparisons. Associations between plasma pool fatty acid measurements and corresponding dietary fatty acid intakes and associations between plasma and erythrocyte n-3 biomarkers were determined by Pearson's correlation coefficients at each time point, and Fisher's exact Z test was used for comparisons of correlations across time.

## **RESULTS**

Fasting blood samples were collected and analyzed for total lipid fatty acid composition in plasma and erythrocytes from 157 African-American women not consuming alcohol during pregnancy at 24 weeks gestation, infant delivery, and 3 months postpartum. Demographic characteristics of the participants at entry into the study at 24 weeks gestation are shown in **Table 1**. Characteristics previously demonstrated to influence fatty acid composition (i.e., smoking, previous deliveries, body mass index, blood glucose) were tested as covariates; however, they had no effect on the findings across time and so were removed from the model.

Selected dietary intakes are shown in **Table 2**. Briefly, the women had higher energy intakes at 24 weeks gestation and at delivery compared with the postpartum intakes. These differences in energy intakes were largely reflected in the absolute intakes of specific nutrients. In terms of percentage of energy, protein and saturated fat intakes did not differ across the three time points. At postpartum, the women reported a significantly lower energy percentage and mass intake of total carbohydrates and a greater energy percentage intake of monounsaturated and polyunsaturated fats. The intakes of DHA and AA in

TABLE 5. Selected maternal plasma and erythrocyte fatty acid ratios at 24 weeks gestation, infant delivery, and 3 months postpartum

	Plasma			Erythrocytes		
Fatty Acid Ratios	24 Weeks Gestation	Delivery	3 Months Postpartum	24 Weeks Gestation	Delivery	3 Months Postpartum
16:0/18:0	$3.7 \pm 0.5^{a}$	$4.0\pm0.7^b$	$2.5\pm0.4^c$	$2.4 \pm 0.2^{a}$	$2.2\pm0.4^b$	$1.8 \pm 0.3^{c}$
DHA/EPA	$11.4 \pm 4.0^{a}$	$12.5 \pm 4.0^{b}$	$3.8 \pm 1.7^{c}$	$23 \pm 13^{a}$	$23 \pm 8^{a}$	$10 \pm 3^{b}$
DPAn-6/AA	$0.07 \pm 0.02^a$	$0.09 \pm 0.02^{b}$	$0.04 \pm 0.01^{c}$	$0.08 \pm 0.02^a$	$0.10 \pm 0.02^{b}$	$0.07 \pm 0.01^{c}$
20-Carbon/22-carbon	$3.4 \pm 0.6^{a}$	$3.3 \pm 0.6^{b}$	$5.7 \pm 1.2^{c}$	$2.6 \pm 0.3^{a}$	$2.5 \pm 0.4^{a}$	$3.6 \pm 0.6^{b}$
DPAn-6/22:4n-6	$1.61 \pm 0.33^a$	$1.83 \pm 0.37^{b}$	$0.96 \pm 0.25^{c}$	$0.25 \pm 0.05^a$	$0.29 \pm 0.07^{b}$	$0.22 \pm 0.05^{c}$
% DHA in total HUFA	$14.6 \pm 2.3^a$	$14.4 \pm 2.1^a$	$9.9 \pm 2.2^{b}$	$15.9 \pm 1.9^a$	$16.1 \pm 2.1^a$	$12.4 \pm 2.0^{b}$
PUFA/MUFA	$1.7 \pm 0.3^{a}$	$1.5 \pm 0.3^{b}$	$2.0 \pm 0.4^{c}$	$1.8 \pm 0.2$	$1.8 \pm 0.2$	$1.7 \pm 0.2$
18:2n-6/18:1n-9	$1.5 \pm 0.3^{a}$	$1.3 \pm 0.3^{b}$	$1.8 \pm 0.4^{c}$	$0.9 \pm 0.1$	$0.8 \pm 0.1$	$1.0 \pm 0.1$
DHA/DPAn-6	$3.6 \pm 1.3^{a}$	$3.0 \pm 0.9^{b}$	$4.1 \pm 1.5^{c}$	$3.9 \pm 1.0^{a}$	$3.5 \pm 0.8^{b}$	$3.6 \pm 1.0^{a,b}$
n-6/n-3	$14.3 \pm 2.6^a$	$14.5 \pm 2.6^a$	$15.3 \pm 2.9^{b}$	$5.1 \pm 0.7^{a}$	$5.2 \pm 0.7^{a}$	$5.8 \pm 0.8^{b}$
n-6 HUFA/n-3 HUFA	$4.6 \pm 0.7^{a}$	$4.7 \pm 0.8^{a}$	$5.2 \pm 0.9^{b}$	$3.5 \pm 0.4^{a}$	$3.5 \pm 0.5^{a}$	$4.0 \pm 0.6^{b}$
Biomarkers of n-3 status						
% n-3 HUFA in total HUFA	$18.3 \pm 2.5^{a}$	$17.9 \pm 2.4^a$	$16.4 \pm 2.7^{b}$	$22.4 \pm 2.1^a$	$22.6 \pm 2.4^{a}$	$20.4 \pm 2.4^{b}$
EPA + DHA (weight %)	$1.94\pm0.47^a$	$1.82 \pm 0.43^{a,b}$	$1.78 \pm 0.46^{b}$	$4.15 \pm 0.59^a$	$4.23 \pm 0.78^a$	$3.58 \pm 0.62^{b}$

Values are means  $\pm$  SD (n = 157) for total lipid extracts. AA, arachidonic acid; DHA, docosahexaenoic acid; DPAn-6, docosapentaenoic acid n-6; EPA, eicosapentaenoic acid. 20-Carbon/22-carbon = (EPA + AA)/(DHA + DPAn-6).

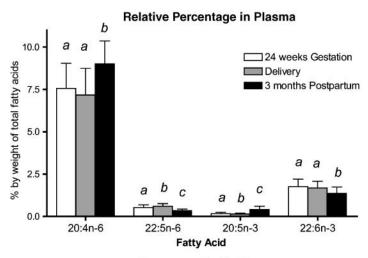
the overall study were  $68 \pm 75$  mg/day (mean  $\pm$  SD) and  $108 \pm 72$  mg/day, respectively, and did not differ statistically at the three time points (Table 2).

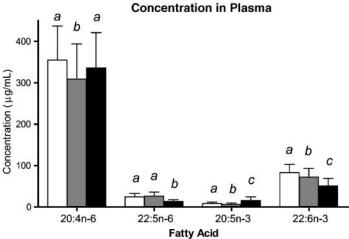
Fatty acid compositions of plasma and erythrocyte total lipid extracts as the weight percentage of total fatty acids are presented in **Tables 3**, **4**. Numerous significant differences were detected in both plasma and erythrocytes, but in general, the differences between the 24 week gestation and delivery time points tended to be smaller in magnitude compared with the differences between these two time points and the postpartum time point. In plasma and erythrocytes, the percentages of DHA and docosapentaenoic acid n-6 (DPAn-6; 22:5n-6) were decreased, whereas EPA and AA were increased postpartum compared with percentage levels at gestation and infant delivery. The percentages of all of the metabolic intermediates between EPA and DHA and also between AA and DPAn-6 were increased in postpartum plasma, whereas in erythrocytes only the weight percentage of docosapentaenoic acid n-3 (DPAn-3; 22:5n-3) was increased postpartum relative to other times. The percentage of individual HUFAs in total plasma HUFAs were also determined and plotted to further demonstrate the changes in the distribution of longchain fatty acids from pregnancy to postpartum (Fig. 1). The percentage of individual HUFAs in total erythrocyte HUFAs exhibited similar results (data not shown). The percentages of AA, EPA, and DPAn-3 in HUFAs were significantly greater, whereas the percentages of DHA and DPAn-6 in HUFAs were significantly less at 3 months postpartum compared with at gestation and delivery. Also, the significant increase in the percentage of DPAn-6 in total HUFAs was significantly greater, and the percentage of EPA in total HUFAs was significantly less, at delivery compared with 24 weeks gestation. Percentages of palmitic acid (16:0) and stearic acid (18:0) responded similarly in both plasma and erythrocytes, with postpartum levels being significantly lower for 16:0 and significantly higher for 18:0. These results and the significantly lower ratios of 16:0/18:0 in plasma and erythrocytes at postpartum (**Table 5**) replicate previous findings (49–51).

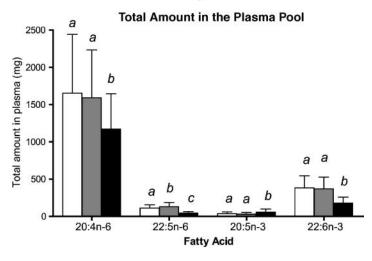
The ratios of selected fatty acids in plasma and erythrocyte total lipid extracts are shown in Table 5. The ratio of DHA to EPA at postpartum was approximately one-third in plasma and one-half in erythrocytes of the DHA/EPA ratios at gestation and delivery. The DPAn-6/AA ratio was significantly lower postpartum than at the other time points in both plasma and erythrocytes and mirrored the DHA/EPA ratio. These results suggest that during pregnancy there are increased percentages of 22-carbon HUFAs in blood that do not persist into the postpartum period. This observation is further supported by the higher ratio of 20-carbon/22-carbon in plasma and erythrocytes at postpartum.

Mean plasma volume was estimated to be  $4.56 \pm 1.37$  L at 24 weeks gestation,  $5.07 \pm 1.52$  L at delivery, and  $3.45 \pm$ 1.03 L at 3 months postpartum. In addition, the total amount of lipid in plasma declines by 41% from delivery to 3 months postpartum. Selected plasma fatty acid percentages, concentrations, and estimates of the total amount of an individual fatty acid in the entire plasma pool (to account for dilution via pregnancy-induced plasma volume expansion) are shown in Fig. 2 (for all fatty acids, see supplementary tables). The concentration of DHA in plasma decreased significantly between 24 weeks gestation (83 ±  $20 \mu g/ml$ ) and delivery (72 ± 21  $\mu g/ml$ ) and decreased further by 3 months postpartum (51  $\pm$  18  $\mu$ g/ml). However, the total amount of DHA in plasma was similar at gestation and delivery (384  $\pm$  162 mg vs. 372  $\pm$  155 mg, respectively) and was decreased postpartum (178  $\pm$  81 mg). This pattern was similar for the total amount in plasma of many of the fatty acids. Interestingly, at 3 months postpartum, the total plasma amounts were higher for  $\gamma$ -linolenic acid (18:3n-6;  $54 \pm 34$  mg postpartum vs.  $42 \pm 20$  mg gestation and 37  $\pm$  21 mg delivery) and EPA (56  $\pm$  42 mg postpartum vs.  $38 \pm 23$  mg gestation and  $33 \pm 19$  mg delivery), and DPAn-3 levels did not change (57  $\pm$  26 mg

 $<sup>^{</sup>a,b,c}$  Means with different superscripts are significantly different across the three time points by Bonferroni post hoc tests after significance was detected by the linear mixed models procedure (P < 0.01).







**Fig. 2.** Amounts of selected fatty acids in plasma expressed as relative percentage of total fatty acids, concentration in plasma, and estimated amount in the total plasma pool (n = 157). Bars with different superscripts within each graph are significantly different across the three time points by Bonferroni post hoc tests after significance was detected by the linear mixed models procedure with the corresponding adjusted intake included as a covariate (P < 0.01). Plasma volume was estimated by determining prepregnancy plasma volume (45 ml of plasma/prepregnant kg) and then adjusting for a 35% increase at 24 weeks gestation, a 50% increase at delivery, and a 2% increase at 3 months postpartum (30, 32).

postpartum,  $54 \pm 23$  mg gestation, and  $54 \pm 22$  mg delivery), despite the physiologic changes that reduce total circulating lipids. Both concentrations and weight percentages of these three fatty acids in plasma were significantly higher at postpartum, whereas the weight percentages of EPA and DPAn-3 were higher in erythrocyte total lipid.

Therefore, between delivery and postpartum, DHA and DPAn-6 decreased in terms of the plasma absolute amount, plasma concentration, and plasma and erythrocyte percentage measures. After delivery, AA decreased in plasma

absolute amount (not in absolute concentration) but increased in terms of the weight percentage in postpartum plasma and erythrocyte. AA concentrations ( $\mu g/ml$ ) in plasma were 13% lower at delivery (309  $\pm$  85  $\mu g/ml$ ) compared with 24 weeks gestation (355  $\pm$  82  $\mu g/ml$ ) and 8% lower postpartum (336  $\pm$  85  $\mu g/ml$ ).

Breast-feeding in this population was low. At delivery and at 3 months postpartum, breast-feeding data were available for 157 and 130 participants, respectively. Using breast-feeding at delivery to categorize the women indi-

cated that 25.5% were breast-feeding (n = 40), and there were no differences in the decrease in the amount of DHA in plasma from delivery to postpartum compared with non-breast-feeding women (n = 117) ( $-204 \pm 134$  mg vs.  $-189 \pm 104$  mg; P = 0.48). Using breast-feeding at 3 months postpartum to categorize the women resulted in 9.2% actively breast-feeding, and there were no differences in the decreases of the amount of DHA in plasma for breast-feeding and non-breast-feeding women (n = 12,  $-178 \pm 107$  mg vs. n = 118,  $-201 \pm 118$  mg; P = 0.53).

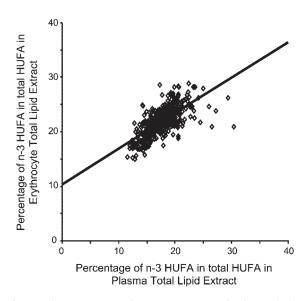
Dietary intakes of individual fatty acids and total amounts of corresponding fatty acids in the plasma pool were examined by correlation analysis (see supplementary tables for complete results). Dietary intakes of total n-3 PUFAs, n-3 HUFAs, EPA, DHA, AA, and n-6 HUFAs were significantly correlated ( $r \ge 0.30$ , P < 0.001 for all) to the corresponding fatty acid amounts in the plasma pool at postpartum but not at 24 weeks gestation and delivery. These correlations were significantly stronger at postpartum compared with gestation and delivery when compared by Fisher's exact Z test.

The percentage of n-3 HUFAs in total HUFAs and the sum of the percentage of EPA plus DHA in both plasma and erythrocytes are presented in Table 5. The coefficient of variance was lowest for n-3 HUFAs/total HUFAs in erythrocytes (11.5%) followed by n-3 HUFAs/total HUFAs in plasma (15.3%) and then by EPA plus DHA in erythrocytes (18.3 weight %) and plasma (24.9 weight %). The correlation between plasma and erythrocyte measures was slightly stronger for the n-3 HUFAs/total HUFAs marker (r=0.70, P<0.001; **Fig. 3**) compared with the EPA plus DHA (weight %) marker (r=0.63, P<0.001) but was not significantly different by Fisher's Z test (P=0.055).

#### DISCUSSION

Plasma lipid and lipoprotein concentrations quickly increase with the onset of pregnancy and rapidly revert to basal levels postpartum (29), although a return to preconception fatty acid status may take up to 6 months (28). This is likely to accommodate the maternal-fetal cumulative accretion of almost 4 kg of total fat during pregnancy (31). In contrast, concentrations of most circulating nutrients decline during pregnancy as a result of a net increase in plasma volume of  $\sim 1.5$  liters (30, 32), even though the total amount of nutrients in the circulation is increased (31). Although fatty acid concentrations increase in early pregnancy, increases in plasma volume continue until  $\sim$ 38 weeks of gestation (52). Therefore, dilution attributable to plasma volume expansion may be an important factor responsible for the decreases in fatty acid concentrations during late pregnancy in addition to the influence of fetal demand. This underlies the need to present fatty acid compositional data as percentages, absolute concentrations, and total amounts in the plasma pool, as is done here.

In the present study, the total amount of DHA in plasma did not decrease from 24 weeks of gestation to in-



**Fig. 3.** Erythrocyte versus plasma percentage of n-3 HUFAs in total HUFAs. Blood samples from gestation, delivery, and postpartum were plotted together (n = 471; r = 0.70, P < 0.001). The means  $\pm$  SD for the percentage of n-3 HUFAs in total HUFAs were 17.5  $\pm$  2.7 with a coefficient of variation of 15.3% for plasma and 21.8  $\pm$  2.5 with a coefficient of variation of 11.5% for erythrocytes.

fant delivery, as indicated by its percentage (1.77  $\pm$  0.44 weight % vs.  $1.68 \pm 0.40$  weight %) and estimated absolute amount in plasma (384  $\pm$  162 mg vs. 372  $\pm$  155 mg). The concentration of DHA in plasma did decrease 13% from 24 weeks of gestation (83  $\pm$  20  $\mu$ g/ml) to delivery (72  $\pm$  21 µg/ml), probably as a result of the normal plasma volume expansion (11% increase) during pregnancy. The absolute amount of DPAn-6 in plasma increased 17%, whereas there were no differences between the absolute amounts of AA, 22:4n-6, and DHA from 24 weeks gestation to delivery. The changes in these longchain fatty acids were examined further by presenting the individual HUFAs as a percentage of total HUFAs (Fig. 1). This figure suggests that DPAn-6 competes with the entire HUFA pool rather than with DHA exclusively and that DPAn-6 accumulation during late pregnancy may possibly be countered by AA percentage decreases rather than reduced DHA status resulting from fetal uptake. The poor utility of plasma DPAn-6 as a biomarker of low DHA status as demonstrated in the present study confirms a recent report by Innis, Vaghri, and King (53).

Other ratios that have been used in past studies (i.e., DPAn-6/22:4n-6 and PUFA/MUFA) do not appear to have utility in indicating DHA and essential fatty acid status, respectively (15), in the present study. These ratios indicate that DHA and essential fatty aid status were at a minimum at delivery. However, the percentage of DHA in total HUFAs is significantly higher at gestation and delivery than at postpartum in both plasma and erythrocytes (Fig. 1), and the PUFA/MUFA ratio largely reflects the ratio of 18:2n-6/18:1n-9 (Table 5). Also, traditional essential fatty acid deficiency indicators, such as 20:3n-9 (54, 55) and the corresponding triene-to-tetraene ratio, are inappropriate in

this population, as 20:3n-9 is largely undetectable (e.g., <0.01%) as a result of the high intake of 18:2n-6 (56). The dietary intake of linoleate in the present population is  $6.9\pm2.0\%$  of total energy (15.5  $\pm$  4.3 g/d or 17.5  $\pm$  3.6% of the energy derived from fat). This is well above the highest experimental linoleate diet (4.9% of total energy) used by Mohrhauer and Holman (57) when they established that a linoleate intake of 1% of total energy corresponds to adequate essential fatty acid status.

Expressing an individual HUFA as a percentage of the total HUFA pool, as done in the present study and described previously (39), is a better indicator of DHA and essential fatty acid status than the other ratios mentioned above. In addition, the use of the percentage of n-3 HUFAs in total HUFAs in either plasma or erythrocyte total lipids appears to be a promising indicator of n-3 fatty acid status, although further data on a range of dietary intakes are needed. A biomarker of n-3 status determined from the total lipid fraction rather than from specific lipid fractions such as phospholipids would reduce the difficulty of developing rapid and fully automated fatty acid analytical procedures.

There is high fetal brain accretion (58) and high placental transfer of both DHA and AA (35, 36). Otto et al. (28) previously proposed that there is an increase in the conversion of DHA from DPAn-3 during pregnancy. The results of the present study support such a hypothesis. The present findings also suggest that the mechanism responsible is not specific for DHA alone and possibly increases the mobilization and/or metabolism of DPAn-6 as well. This would be consistent with a shared elongation and desaturation pathway, possibly involving the peroxisome, and is supported by the observed decrease in weight percentage of EPA and AA during pregnancy. Such a metabolic adaptation would increase the relative amount of DHA available for placental transfer. Although it also has the potential to reduce the availability of AA, this is unlikely, as AA accounts for >60% of total HUFAs in the circulation of the women in the present study (Fig. 1). The absolute amounts of AA and DHA in plasma did not differ during the second half of pregnancy but were lower postpartum (Fig. 2).

These differences in fatty acids may be the result of hormonal changes during pregnancy and/or postpartum. Women of different hormonal status have different plasma phospholipid fatty acid profiles (59), and hormone replacement therapy has been associated with differences of EPA and DHA amounts in plasma phospholipids (60). Studies using fatty acid stable isotopes have reported that women of childbearing age have a greater potential to produce DHA than men (61, 62). In addition, Giltay et al. (63) recently demonstrated that higher DHA concentrations in women compared with men are likely because of estrogenic effects. Therefore, the observed decrease of circulating DHA at postpartum is likely to be hormonally mediated, although lactational demands for DHA may make a contribution (27, 28). However, in the present study, there were no differences in the decrease of DHA from delivery to 3 months postpartum between breast-feeding (n = 12,  $-178 \pm 107$  mg) and non-breast-feeding (n = 118,  $-201 \pm 118$  mg) women.

This is the largest study to date to determine dietary intakes of specific fatty acids in pregnant women, particularly for DHA and AA. DHA intake in the overall study was 68 ± 75 mg/day and is slightly higher but similar to that reported in low-income pregnant women from Nebraska (64) and lower than estimates from inner-city pregnant women from Memphis (65). The present DHA estimate is lower than DHA estimates of 160 mg/day (66) and 97 mg/day (67) in pregnant Canadians, 140 mg/day in pregnant Mexicans (68), and 140 mg/day (24) and 300 mg/day (16) in pregnant Europeans. In contrast, nonpregnant Japanese women consume 571 mg/day DHA (69), reflecting the high amount of seafood in their diets.

In the present study of pregnant women consuming very low amounts of n-3 HUFAs, there were only weak correlations during pregnancy between specific dietary HUFAs and the corresponding HUFAs in plasma, in contrast to strong correlations postpartum. Pawlosky et al. (62) demonstrated that the conversion of DPAn-3 to DHA is higher in women of childbearing age while on a beef-based diet (low n-3 HUFAs) versus a fish-based diet (high n-3 HUFAs). During pregnancy in the present population, there may have been metabolic adaptations that served to increase the availability of DHA in the maternal circulation for placental transfer. This adaptation may not occur in women consuming higher amounts of DHA. Studies with carefully quantified dietary fatty acid intakes and using stable isotopes are needed to further investigate possible hormone-mediated changes in fatty acid metabolism during pregnancy.

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Supplementary Table 1. Plasma Fatty Acid Composition at 24 weeks Gestation, Infant Delivery and Three months Postpartum Expressed as Concentrations <sup>1</sup>

	Concentration $(\mu g/mL)$				
Fatty acids	24 wks Gestation	Delivery	3 months Postpartum		
	(n = 157)	(n = 157)	(n = 157)		
SFA <sup>2</sup>	$1481 \pm 327^{a}$	$1410 \pm 409^{a}$	$1158 \pm 275$ b		
$14:0^2$	$44\pm22^{a}$	$31 \pm 15^{b}$	$29 \pm 14^{b}$		
$16:0^2$	$1085 \pm 258^{a}$	$1060 \pm 328^{a}$	$766 \pm 199^{\mathrm{b}}$		
$18:0^2$	$295 \pm 56^{a}$	$266 \pm 71$ b	$308\pm70^{\mathrm{\ a}}$		
20:0	$10.7 \pm 4.0^{\text{ a}}$	$8.8 \pm 2.3^{\text{ b}}$	$7.9 \pm 2.0^{\rm c}$		
22:0	$21.1 \pm 5.4^{a}$	$23.9 \pm 6.4^{\text{ b}}$	$24.5 \pm 6.2^{\text{ b}}$		
24:0	$17.0 \pm 5.6^{a}$	$17.9 \pm 5.0^{ab}$	$19.0 \pm 4.7^{\mathrm{b}}$		
$MUFA^2$	$1154 \pm 296^{a}$	$1141 \pm 381^{a}$	$816 \pm 262^{\text{ b}}$		
$16:1n-7^2$	$101\pm45^{\mathrm{a}}$	$88 \pm 43$ b	$65 \pm 29^{\circ}$		
18:1n-7	$108\pm27^{a}$	$98 \pm 30^{ b}$	$86 \pm 27^{\mathrm{c}}$		
$18:1n-9^2$	$850 \pm 230^{\text{ a}}$	$873 \pm 305^{a}$	$590 \pm 200^{\mathrm{b}}$		
$20:1n-9^2$	$7.7 \pm 3.6^{a}$	$9.8 \pm 4.1^{\text{ b}}$	$6.3 \pm 2.3^{\text{ c}}$		
22:1n-9	$2.2 \pm 1.8^{a}$	$1.8 \pm 0.6^{\ b}$	$2.7 \pm 1.0^{a}$		
24:1n-9	$79\pm36^{\mathrm{a}}$	$69 \pm 24^{\rm b}$	$62 \pm 32^{b}$		
Total PUFA <sup>2</sup>	$1874 \pm 372^{a}$	$1647 \pm 424^{b}$	$1580 \pm 359^{b}$		
n-6 PUFA <sup>2</sup>	$1749 \pm 354^{a}$	$1538 \pm 400^{\text{ b}}$	$1480 \pm 336^{\ b}$		
$18:2n-6^2$	$1262 \pm 289^{a}$	$1105 \pm 313^{b}$	$1031 \pm 252^{b}$		
18:3n-6	$9.2 \pm 3.9^{a}$	$7.5 \pm 3.8^{\text{ b}}$	$15.3 \pm 8.0^{\circ}$		
20:2n-6	$12.9 \pm 3.3^{a}$	$11.6 \pm 3.9^{b}$	$7.8 \pm 2.5^{\text{ c}}$		
20:3n-6	$69 \pm 18^{a}$	$64\pm20^{ab}$	$62 \pm 20^{\mathrm{b}}$		
$20:4n-6^2$	$355 \pm 82^{a}$	$309 \pm 85^{b}$	$336 \pm 85^{a}$		
22:2n-6	$0.94 \pm 0.40$	$1.00 \pm 0.47$	$1.40 \pm 0.99$		
22:4n-6	$15.4 \pm 4.3^{a}$	$14.1 \pm 4.1^{\text{ b}}$	$13.8 \pm 3.9^{b}$		
$22:5n-6^2$	$24.6 \pm 8.1^{a}$	$26.1 \pm 9.9^{a}$	$13.0 \pm 4.6^{b}$		
n-6 HUFA <sup>2</sup>	$465 \pm 98^{a}$	$414 \pm 110^{b}$	$425 \pm 103^{\ b}$		
n-3 PUFA <sup>2</sup>	$125\pm28^{a}$	$108 \pm 31^{\ b}$	$100 \pm 32^{\ b}$		
$18:3n-3^3$	$21.2 \pm 8.1^{a}$	$18.5 \pm 7.9^{\text{ b}}$	$16.1 \pm 7.1^{\text{ c}}$		
20:3n-3	$0.91 \pm 0.40^{\rm \ a}$	$0.73 \pm 0.34^{b}$	$1.73 \pm 1.08^{\circ}$		
$20:5n-3^2$	$8.2 \pm 3.6^{a}$	$6.4 \pm 3.0^{\text{ b}}$	$15.6 \pm 8.5^{\text{ c}}$		
22:5n-3	$11.9 \pm 3.6^{a}$	$10.7 \pm 3.3^{\ b}$	$16.5 \pm 5.4^{\circ}$		
22:6n-3 <sup>2</sup>	$83\pm20^{a}$	$72 \pm 21$ b	$51 \pm 18^{c}$		
n-3 HUFA <sup>2</sup>	$104\pm24^{a}$	$90 \pm 26^{b}$	$84\pm27^{\text{ b}}$		
Total HUFA <sup>2</sup>	$568 \pm 117^{a}$	$504 \pm 132^{b}$	$510 \pm 125^{b}$		
Total <sup>2</sup>	$4956 \pm 977^{a}$	$4414 \pm 1210^{b}$	$3761 \pm 908^{\circ}$		

<sup>7</sup>Values are means  $\pm$  SD for the plasma total lipid extracts. Means with different superscripts are significantly different across the three time points by Bonferroni post hoc tests after significance detected by Linear Mixed Models procedure, P < 0.01. <sup>2</sup>Corresponding adjusted dietary intake included as a covariate in the model with no changes in the statistical results. <sup>3</sup>Including dietary 18:3n-3 intake as a covariate resulted in no difference between the estimated marginal means of the concentration of 18:3n-3 at infant delivery and three months Postpartum by Bonferroni post hoc tests. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; HUFA, highly unsaturated fatty acids (≥20 carbons and ≥3 double-bonds).

Supplementary Table 2. Plasma Fatty Acid Composition at 24 weeks Gestation, Infant Delivery and Three months Postpartum Expressed as Concentrations<sup>1</sup>

	Total amount in plasma (mg)				
Fatty acids	24 wks Gestation	Delivery	3 months Postpartum		
	(n = 157)	(n = 157)	(n = 157)		
SFA <sup>2</sup>	6813 ± 2579 a	$7086 \pm 2567^{\text{ a}}$	$4032 \pm 1507^{\text{ b}}$		
$14:0^2$	$197 \pm 105^{a}$	$151 \pm 75^{\text{ b}}$	$100 \pm 60^{\circ}$		
$16:0^2$	5001 ± 1975 a	$5329 \pm 2011^{a}$	$2666 \pm 1011^{b}$		
$18:0^2$	$1354 \pm 488^{a}$	$1337 \pm 464^{a}$	$1072 \pm 413^{b}$		
20:0	$48\pm22^{\mathrm{a}}$	$44 \pm 15^{a}$	$27 \pm 11^{b}$		
22:0	$95 \pm 36^{a}$	$121 \pm 45^{b}$	$84 \pm 34^{c}$		
24:0	$76 \pm 31^{a}$	$90 \pm 36^{b}$	$65\pm27^{\rm c}$		
$MUFA^2$	$5306 \pm 2107^{a}$	$5735 \pm 2210^{a}$	$2847 \pm 1205^{b}$		
$16:1n-7^2$	$467 \pm 254^{a}$	$437 \pm 220^{a}$	$228 \pm 124^{\text{ b}}$		
18:1n-7	$495 \pm 189^{a}$	$494 \pm 178^{a}$	$296 \pm 118^{b}$		
$18:1n-9^2$	$3914 \pm 1609^{a}$	$4394 \pm 1758$ b	$2059 \pm 886^{\text{ c}}$		
$20:1n-9^2$	$35\pm20^{\mathrm{a}}$	$49 \pm 21^{b}$	$22 \pm 10^{\circ}$		
22:1n-9	$10 \pm 8$	$9 \pm 4$	$9 \pm 4$		
24:1n-9	$356 \pm 188^{a}$	$347 \pm 155^{a}$	$219 \pm 138^{b}$		
Total PUFA <sup>2</sup>	$8539 \pm 3077^{a}$	$8332 \pm 2933$ a	$5482 \pm 1994^{b}$		
n-6 PUFA <sup>2</sup>	$7964 \pm 2883^{\text{ a}}$	$7779 \pm 2740^{a}$	$5131 \pm 1859^{b}$		
$18:2n-6^2$	$5707 \pm 2042^{a}$	$5563 \pm 2034^{a}$	$3564 \pm 1290^{\text{ b}}$		
18:3n-6	$42\pm20^{\mathrm{\ a}}$	$37 \pm 21^{a}$	$54 \pm 34^{c}$		
20:2n-6	$58 \pm 21^{\text{ a}}$	$58\pm23^{\mathrm{a}}$	$27 \pm 12^{b}$		
20:3n-6	$317 \pm 131^{a}$	$323 \pm 132^{a}$	$218 \pm 103^{b}$		
$20:4n-6^2$	$1655 \pm 788^{a}$	$1591 \pm 644^{a}$	$1173 \pm 471^{\text{ b}}$		
22:2n-6	$4\pm2$	$5\pm2$	$5\pm4$		
22:4n-6	$71 \pm 29^{a}$	$72\pm29^{\mathrm{a}}$	$48 \pm 21^{\ b}$		
$22:5n-6^2$	$112 \pm 44^{a}$	$131 \pm 55^{b}$	$45 \pm 18^{c}$		
n-6 HUFA <sup>2</sup>	$2157 \pm 952^{a}$	$2120 \pm 827^{a}$	$1486 \pm 590^{\text{ b}}$		
n-3 PUFA <sup>2</sup>	$575 \pm 224^{a}$	$553 \pm 220^{a}$	$351 \pm 158^{b}$		
18:3n-3	$95 \pm 42^{a}$	$92 \pm 44^{a}$	$56 \pm 28^{b}$		
20:3n-3	$4\pm2^{a}$	$4\pm2^{a}$	$6\pm4^{\rm \ b}$		
$20:5n-3^2$	$38\pm23^{\mathrm{\ a}}$	$33 \pm 19^{a}$	$56 \pm 42^{\text{ b}}$		
22:5n-3	$54 \pm 23$	$54 \pm 22$	$57 \pm 26$		
22:6n-3 <sup>2</sup>	$384 \pm 162^{a}$	$372 \pm 155^{a}$	$178 \pm 81^{\ b}$		
n-3 HUFA <sup>2</sup>	$480 \pm 198^{a}$	$462 \pm 191^{a}$	$296 \pm 138^{b}$		
Total HUFA <sup>2</sup>	$2637 \pm 1132^{a}$	$2582 \pm 1004$ a	$1785 \pm 714^{\text{ b}}$		
Total <sup>2</sup>	$22712 \pm 8193^{a}$	$22235 \pm 7810^{a}$	$13079 \pm 4862^{b}$		
Plasma volume estimate (L)	$4.56 \pm 1.37^{a}$	$5.07 \pm 1.52^{\text{ b}}$	$3.45\pm1.03^{\circ}$		

<sup>1</sup>Values are means  $\pm$  SD for the plasma total lipid extracts. Means with different superscripts are significantly different across the three time points by Bonferroni post hoc tests after significance detected by Linear Mixed Models procedure, P < 0.01. Plasma volume estimated by determining pre-pregnancy plasma volume (45 mL of plasma/pre-pregnant kg) and then adjusting for a 35% increase at 24 wks gestation, a 50% increase at delivery and a 2% increase at three months postpartum (30,32). <sup>2</sup>Corresponding adjusted intake included as a covariate in the model with no changes in the statistical results. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; HUFA, highly unsaturated fatty acids (≥20 carbons and ≥3 double-bonds).

Supplementary Table 3. Correlations of Adjusted Dietary Intakes of Selected Fatty Acids to the Corresponding Fatty Acids Total Amounts in the Plasma Pool of Pregnant African-American Women at 24

weeks Gestation, Infant Delivery and Three months Postpartum

Dietary (g/day) to	24 wks	Gestation	Deli	very	3 months	Postpartum
Plasma (mg)	(n = 157)		(n = 157)		(n = 157)	
	r	P	r	P	r	P
Total Fatty Acids	-0.02	0.81	-0.17	0.04	0.01	0.91
SFA	0.01	0.87	-0.20	0.02	-0.07	0.43
14:0	0.07	0.37	-0.10	0.22	0.04	0.61
16:0	-0.01	0.87	-0.20	0.02	-0.07	0.42
18:0	0.07	0.43	-0.15	0.07	-0.01	0.92
MUFA	-0.002	0.98	-0.11	0.21	0.05	0.58
16:1n-7	0.05	0.59	-0.08	0.32	0.16	0.05
18:1n-9	-0.01	0.91	-0.09	0.27	0.01	0.94
20:1n-9	-0.02	0.84	0.08	0.33	0.20	0.013
PUFA	-0.01	0.87	-0.07	0.40	0.06	0.45
n-6 PUFA	-0.02	0.83	-0.07	0.43	0.05	0.59
18:2n-6	0.02	0.81	-0.05	0.59	0.06	0.49
20:4n-6	$0.04^{a}$	0.62	$0.04^{a}$	0.64	0.30 <sup>b</sup>	< 0.001
22:5n-6	-0.04	0.62	-0.02	0.84	0.17	0.04
n-6 HUFA	0.05 <sup>a</sup>	0.59	$0.04^{a}$	0.64	$0.32^{\mathrm{b}}$	< 0.001
n-3 PUFA	0.01 <sup>a</sup>	0.94	$0.004^{a}$	0.96	$0.32^{b}$	< 0.001
18:3n-3	0.07	0.37	-0.02	0.85	0.20	0.02
20:5n-3	0.12 a	0.17	$0.12^{a}$	0.17	$0.48^{\mathrm{b}}$	< 0.001
22:6n-3	0.13 <sup>a</sup>	0.12	$0.07^{a}$	0.43	$0.43^{b}$	< 0.001
n-3 HUFA	0.11 a	0.19	$0.06^{a}$	0.48	$0.47^{\mathrm{b}}$	< 0.001

Dietary and plasma fatty acids were correlated by Pearson's coefficient. Dietary values are adjusted for energy by the nutrient residual model (43). Correlations with P < 0.01 are considered significant (2-tailed). Correlations with different superscripts are significantly different across time points by Fisher's Exact Z-test, P < 0.01. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; HUFA, highly unsaturated fatty acids ( $\ge 20$  carbons and  $\ge 3$  double-bonds).